ORIGINAL ARTICLE

# Multiresistant *Staphylococcus epidermidis* in a neonatal care unit

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**Objective:** To study the presence and diversity of types of *Staphylococcus epidermidis* in the neonatal intensive care unit of a university hospital.

**Methods:** During a period of 6 weeks, samples were taken from nose, external auditory canal, axilla, groin and umbilicus from consecutively admitted patients. Patients were sampled two times a week for up to 2 weeks. Isolates of *S. epidermidis* were characterized by antibiogram, plasmid pattern and biotype.

**Results:** Fifteen patients were included. Each patient was sampled in one to four successive surveys, depending on the admission period. A total of 128 isolates of *S. epidermidis* were obtained and allocated to seven antibiogram types, 36 plasmid types and 14 biotypes. One plasmid type found in 58 isolates (six patients) corresponded with one multiresistant antibiogram type. The number of isolates with these characteristics increased per neonate from the first survey to the fourth. Nineteen isolates from four patients were allocated to a second plasmid type and were of a common antibiogram type. The remaining 34 plasmid types were sporadic. No clear correspondence of biotypes with antibiogram or plasmid types was found.

**Conclusions:** The present study revealed the increase in colonization of a multiresistant type of *S. epidermidis* in the compromised patients during admission to the ward. Further studies have to assess whether this type remains persistent in the ward.

**Key words:** *Staphylococcus epidermidis*, multiresistant, neonatal intensive care unit, plasmid typing, antibiogram typing, biotyping

## INTRODUCTION

*Staphylococcus epidermidis* can be involved in life-threatening infections in neonates, particularly in those with low birth weight or intravascular catheters [1, 2]. Several studies have indicated that these infections are associated with specific strains that have spread in neonatal care units [3,4]. *S. epidermidis* is the most common coagulase-negative staphylococcus species in neonates [5]. The mode of spread of this organism is not fully understood because there is not complete agreement concerning the best typing system and the intercorrelation of results.

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In the present investigation the colonization of neonates with *S. epidermidis* during their stay in the neonatal intensive care unit (NICU) of the Leiden University Hospital was studied with the aim of establishing whether particular strains predominated in the ward. Fifteen patients were sampled repeatedly over a period of up to 2 weeks. Isolates identified as *S. epidermidis* were compared by antibiogram, plasmid profile and biotype.

## PATIENTS, MATERIALS AND METHODS

The study was performed at the neonatal intensive care unit (NICU) of the Leiden University Hospital. The ward has nine incubators and usually a nurse is assigned to each neonate.

### Sampling

Fifteen patients consecutively admitted in the period from 9 March to 16 April 1993 were included. Each

patient was sampled during a period of up to 2 weeks, depending on the duration of admission, each Tuesday and Friday. Samples were taken from nose, external auditory canal, axilla, groin and umbilicus. The swabs were inoculated onto blood agar (CM271, Oxoid, Basingstoke, Hampshire, UK) enriched with 5% sheep blood and incubated for 48 h at 35 °C.

### Identification

The plates were inspected for different morphotypes, and colonies representative for each type were analyzed further. Isolates were identified as *S. epidermidis* on the basis of the following criteria: Gram-positive cocci, catalase and phosphatase positive, coagulase and oxidase-negative, susceptible to furazolidone and novobiocin and producing acid from maltose and sucrose but not from trehalose [6,7].

### Antibiogram determination

The antibiogram was determined by disk-diffusion on Iso-Sensi test agar (CM473, Oxoid), according to the Dutch National Standards [8]. The antibiotic disks used were penicillin (10 μg), methicillin (10 μg), vancomycin (30 μg), erythromycin (15 μg), clindamycin (10 μg), rifampicin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), trimethoprim (1.25 μg), fusidic acid (10 μg) and ciprofloxacin (5 μg). Methicillin susceptibility was assayed by disk-diffusion on 5% sheep blood agar for 48 h at 30 °C. Disks were obtained from Becton Dickinson (Cockeysville, Maryland, USA), except for methicillin and clindamycin, which were from Oxoid. The panel of antibiotics, containing different classes of antibiotics, could be expected to discriminate between isolates.

For typing, the unweighted values of the diameters of the inhibition zones were subjected to cluster analysis with the SPSS statistical software package [9]. Squared euclidian distances were calculated between all possible pairs of isolates and clusters were generated by Ward's method [10].

### Plasmid typing

Plasmid DNA was extracted as described by Parisi and Hecht [11] with minor modifications. Briefly, a loopful of cells from overnight cultures on 5% sheep blood agar was lysed by a 50 mM Tris-EDTA solution containing 1% Brij-58 (P5885, Sigma) and 0.4% sodium deoxycholate. After centrifugation (12,500 *g*) for 1 h, 100 μl of the supernatant was mixed with 10 μg RNase (109169, Boehringer Mannheim) and incubated for 30 min at 30 °C. Proteolysis was performed by adding 10 μg proteinase K (11048E, Gibco BRL, Gaithersburg, USA) and subsequent incubation for 30 min at 37 °C. DNA was precipitated by adding 240 μl 96% ethanol and storing for 18 h at −20 °C. After centrifugation (12,500 *g*) for 10 min, the pellet was dissolved in 15 μl distilled water. The samples were mixed with 10 μl loading buffer (33% glycerol, 0.05% bromophenol blue in distilled water) and were electrophorized horizontally in 0.7% agarose gel with 0.5×TBE buffer at 100 V constant voltage for 2.5 h. Plasmids of *Escherichia coli* 39RB61 [12] and *E. coli* V517 [13] were included as a reference for plasmid size determination.

**Table 1.** Characteristics of antibiograms (median zone diameter and range in millimeters) of isolates in each antibiogram cluster

| Antibiogram cluster | Number of isolates | Antibiotic | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PEN | MET | VAN | ER | CLI | RIF | CHL | GEN | TRI | FUS | CIP |
| A | 58 | 21 | 5 | 23 | 5 | 5 | 42 | 6 | 5 | 5 | 37 | 33 |
| | | (18–32) | ( 5–22) | (21–30) | – | – | (37–50) | ( 5–11) | ( 5–15) | ( 5–17) | (21–44) | (30–39) |
| B | 20 | 25 | 11 | 23 | 36 | 42 | 44 | 30 | 5 | 5 | 37 | 32 |
| | | (20–31) | ( 5–20) | (20–26) | (34–40) | (39–47) | (41–49) | (27–34) | ( 5–12) | ( 5–12) | (34–41) | (30–35) |
| C | 20 | 26 | 29 | 22 | 35 | 40 | 41 | 31 | 35 | 28 | 36 | 34 |
| | | (20–42) | ( 5–35) | (21–26) | (11–40) | (31–50) | (38–48) | (29–38) | (31–40) | (20–38) | ( 9–48) | (27–39) |
| D | 14 | 19 | 20 | 22 | 35 | 42 | 42 | 31 | 7 | 26 | 37 | 32 |
| | | (16–25) | ( 5–24) | (21–25) | (34–40) | (38–45) | (40–47) | (30–34) | ( 5–12) | (23–33) | (15–42) | (29–34) |
| E | 7 | 5 | 5 | 25 | 38 | 41 | 48 | 5 | 6 | 5 | 42 | 34 |
| | | ( 5–22) | ( 5–25) | (23–28) | (32–40) | (39–44) | (42–50) | ( 5– 8) | ( 5– 7) | – | (34–45) | (33–37) |
| F | 6 | 26 | 5 | 22 | 5 | 40 | 42 | 5 | 14 | 5 | 37 | 30 |
| | | (23–28) | ( 5–29) | (20–23) | – | (38–43) | (40–43) | ( 5–10) | (11–14) | ( 5–10) | (35–40) | (26–30) |
| G | 3 | 25 | 15 | 23 | 37 | 41 | 5 | 32 | 20 | 24 | 38 | 34 |
| | | (17–26) | (15–29) | (21–24) | (36–39) | (40–43) | – | (31–33) | (20–34) | (19–25) | (38–39) | (33–36) |

Clusters were determined by cluster analysis based on zone sizes using squared euclidian distance and Ward's criterium for clustering. The cutting level for delineating clusters A to G was at a rescaled distance of 2/25 of the dendrogram (not shown).
PEN = penicillin; MET = methicillin; VAN = vancomycin; ER = erythromycin; CLI = clindamycin; RIF = rifampicin; CHL = chloramphenicol; GEN = gentamicin; TRI = trimethoprim; FUS = fusidic acid; CIP = ciprofloxacin.

### Biotyping

API ID32 (BioMérieux, Marcy l'Etoile, France) was used for differentiation below species level. Each different code was considered a biotype.

## RESULTS

### Patients

Fifteen patients were investigated, 11 of whom received one or more antibiotics for clinically suspected infections. Eight patients received the combination amoxicillin–gentamicin, the standard empirical treatment for suspected septicemia on the NICU. None of the suspected infections was confirmed by the microbiological laboratory.

### Samples

A total number of 275 isolates of coagulase-negative staphylococci were obtained from the 15 patients. Of these, 128 (47%) were identified as *S. epidermidis*. The remaining 147 isolates were not identified further. Fifteen patients were sampled in a first survey, ten patients were left in a second and third survey, and five were left in a fourth survey. The number of isolates of *S. epidermidis* per patient ranged from 0 to 19 (median 9). The number of body sites per patient positive for *S. epidermidis* was 0 to 4 (mean 1.3) in the first survey, 0 to 4 (mean 2.5) in the second, 1 to 5 (mean 3.2) in the third, and 0 to 5 (mean 3.8) in the fourth. The *S. epidermidis* isolates were obtained from umbilicus or umbilical cord ($n$ = 18), nose

**Table 2.** Features of each antibiogram cluster within 128 isolates of *S. epidermidis*

| Susceptibility type | | API ID-32 code | Plasmid pattern (no. of each type) | Patient code |
|---|---|---|---|---|
| Code | Main feature | | | |
| A | Multiresistant[a] | 166032200 | *p1* (18) | 2; 3; 10; 11 |
| | | 166032210 | *p1* (31) | 2; 3; 5; 8; 10; 11 |
| | | 167032210 | *p1* (7) | 8; 10; 11 |
| | | 167033210 | *p1* (1) | 3 |
| | | 366032200 | *p1* (1) | 3 |
| B | GEN-resistant; TRI-resistant | 366032200 | *p2* (5); *p3* (1) | 7; 8; 12 |
| | | 366032210 | *p2* (8) | 2; 7; 8 |
| | | 367032210 | *p2* (6) | 7; 11 |
| C | Susceptible[b] | 166032200 | *p19* (1) | 15 |
| | | 166032210 | *p5* (1); *p7* (1); *p8* (1); *p9* (1) | 4; 15 |
| | | 167032200 | *p21* (2) | 13 |
| | | 167032210 | *p10* (1); *p13* (1) | 4 |
| | | 366030210 | *p6* (1) | 15 |
| | | 366032010 | *p17* (1) | 4 |
| | | 366032200 | *p4* (1); *p14* (1) | 1; 5 |
| | | 366032210 | *p11* (1); *p12* (1); *p18* (1); *p20* (2) | 4; 14 |
| | | 367032210 | *p15* (1); *p16* (1) | 9; 13 |
| D | GEN-resistant | 166012000 | *p29* (1) | 9 |
| | | 166032200 | *p22* (1); *p23* (1); *p25* (1); *p30* (1) | 2; 12 |
| | | 166032210 | *p30* (1) | 2 |
| | | 167032020 | *p31* (1) | 4 |
| | | 167032210 | *p31* (1) | 4 |
| | | 366032200 | *p24* (1); *p27* (1) | 2; 12 |
| | | 366032210 | *p26* (1); *p28* (1); *p29* (2) | 2; 8; 9 |
| E | GEN-resistant; TRI-resistant; CHL-resistant | 166032210 | *p32* (1) | 4 |
| | | 167032210 | *p20* (1); *p33* (5) | 4; 10; 15 |
| F | ER-resistant | 166032200 | *p34* (3) | 15 |
| | | 166032210 | *p34* (2) | 15 |
| | | 367032210 | *p34* (1) | 15 |
| G | RIF-resistant | 167030200 | *p33* (1) | 15 |
| | | 366030200 | *p35* (1) | 14 |
| | | 366030210 | *p36* (1) | 15 |

CHL = chloramphenicol; ER = erythromycin; GEN = gentamicin; RIF = rifampicin; TRI = trimethoprim.

[a]With the exception of two strains at least resistant to penicillin, methicillin, erythromycin, clindamycin, chloramphenicol, gentamicin and trimethoprim.

[b]With the exception of penicillin, one isolate resistant to methicillin, one isolate resistant to erythromycin and three isolates resistant to fusidic acid.

($n$ = 22), groin ($n$ = 26), axilla ($n$ = 29) and external auditory canal ($n$ = 33).

## Typing

### *Antibiogram typing*

Isolates were grouped according to their similarity in antibiogram by cluster analysis of the diameters of inhibition zones. Seven clusters (A to G) were distinguished in the dendrogram (not shown) at a relative dissimilarity level of 8%. Tables 1 and 2 show characteristics of isolates in the clusters. Cluster A (type A) comprised 58 isolates (45%) from six patients. These were, except for two isolates, resistant to penicillin, methicillin, erythromycin, clindamycin, chloramphenicol, gentamicin and trimethoprim. In contrast, cluster C (type C) comprised 20 isolates (16%) from seven patients that were sensitive to most antibiotics tested. Characterization of each of the other clusters is shown in Table 2.

### *Plasmid profiling*

Each of the 128 isolates showed one or several plasmid bands, allowing 36 different patterns to be distinguished in total. With exclusion of the chromosomal band, the number of plasmid bands ranged from one to seven (2.1 to 54.6 kb) per pattern. One plasmid type, *p1*, with five bands in the 2.4 to 22.7 kb range was observed in 58 isolates from six patients.

A complete correspondence was found for isolates with plasmid type *p1* and isolates of the antibiogram type A (Table 2). Another plasmid type, *p2*, contained five bands in the 2.0–53.0-kb range. This type was observed in 19 isolates which were all of antibiogram type B (Table 2). Complete correspondence was also found between plasmid type *p34* and antibiogram type F, which were found in six isolates from one patient. The other 34 plasmid patterns (*p3* to *p33* and *p35* to *p36*) occurred sporadically and no clear correspondence was found with any of the antibiogram clusters (Table 2). Figure 1 shows the pattern of plasmid type *p1* and of several sporadic plasmid types.

Isolates with plasmid pattern *p1* were cultured from six patients. In each survey, type *p1* was isolated from four neonates, indicating a relative increase of type *p1* in time as the total number of patients sampled decreased during the four surveys. A similar increase

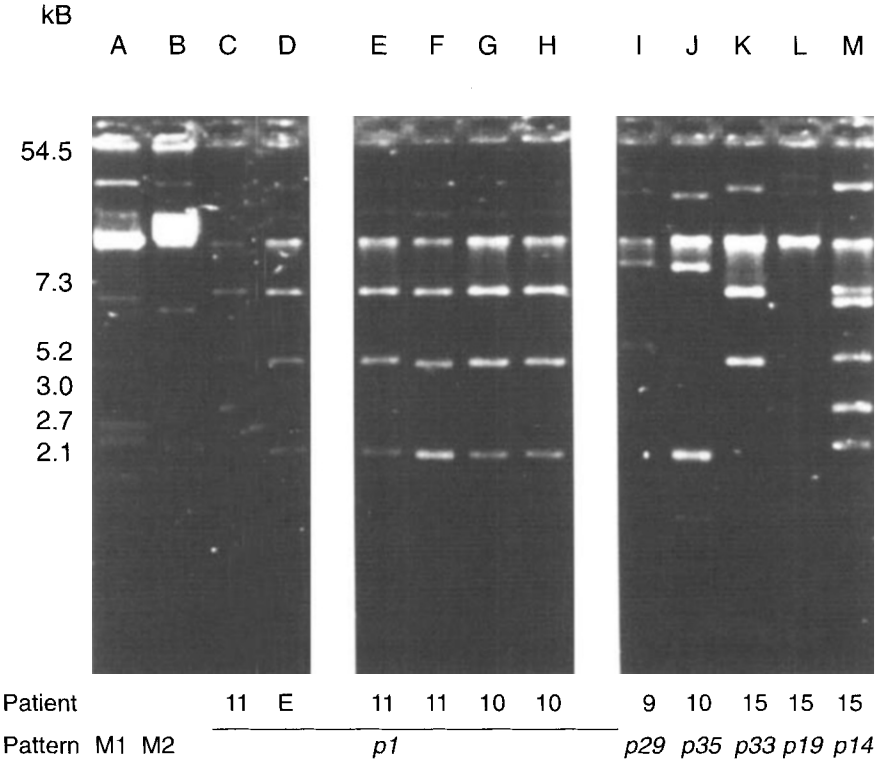


**Figure 1** Plasmid patterns from *Staphylococcus epidermidis* isolates from neonatal intensive care patients. Lanes A and B: marker strains. M1 = *E. coli* V517, M2 = *E. coli* 39RB61. Lanes C to H: plasmid type *p1* found within the predominant strain. Lanes I to M: sporadic patterns.

was not observed for other plasmid patterns. The mean time of admission on the ward before the first survey of patients with isolates of type *p1* in the first survey was 3.5 days (range 2 to 4), whereas the mean admission time was 2.3 days (range 1 to 4) in those cases where only isolates of another type were cultured. No correlation of a specific plasmid pattern or antibiogram type with body site or incubator was observed.

### *Biotyping*

Fourteen API ID32 codes were distinguished within the 128 isolates and compared with antibiogram and plasmid pattern. The slightly different codes 166032200 and 166032210 were found in 65 isolates, 49 of which were of plasmid type *p1*. The remaining nine isolates of plasmid type *p1* were allocated to three other API ID32 codes. The 19 isolates of plasmid type *p2* could be allocated to the codes 366032200, 366032210 and 367032210 observed in 37 isolates. However, because the codes mentioned were also observed in sporadic isolates, there was no strict correlation between API ID32 codes and plasmid pattern or antibiogram type. No association was observed between API ID32 code and survey number, body site or incubator.

Apart from API, a microsystem of 345 phenotypical tests, comprising carbon source utilization, sugar fermentation and enzyme tests, was explored [14]. Due to poor growth, tests were difficult to score and further use was abandoned.

## DISCUSSION

In this short survey of colonization with *S. epidermidis* of 15 patients in a NICU, it is shown that although multiple types were distinguished, two types were predominant as shown by antibiogram and by plasmid typing.

Six out of the 15 neonates studied became colonized with the predominant multiresistant *S. epidermidis* strain. The proportion of isolates belonging to this type increased from 9/25 in the first survey to 22/30 in the fourth survey. This suggests that the patients were increasingly colonized by the multiresistant strain during their stay on the ward. This suggestion is strengthened by the observation that patients with isolates of type *p1* in the first survey had a mean time of admittance of 3.5 days before the first survey was performed, whereas patients with any other type had an admittance time of 2.3 days. Although during the present study clinical infections caused by the predominant types were not observed, the observations by Carlos et al [3] and by Huebner et al [4] that bacteremia in neonates is caused by certain specific virulent strains clearly indicate that colonization with specific types occurs prior to, and probably is a prerequisite for, infection.

Although the 128 isolates were divided into 14 biotypes with the API ID32, the difference in coding was mostly based on one test only. Some of these reactions were difficult to score, and therefore did not result in unambiguous discrimination between isolates. Other studies have reported not only similar results but also poor reproducibility on repeated testing [3,15,16]. Therefore, biotyping with API does not seem a first method of choice.

Neonates in the Leiden University Hospital suspected of having septicemia are empirically treated with the combination amoxicillin–gentamicin, and 8 out of the 15 patients investigated were treated with this combination. In the present study 105 out of 128 isolates, including the predominant types, were resistant or intermediately resistant to gentamicin, which may be the result of the antibiotic use in the ward [17]. Further studies are required to establish whether predominant types are persistent and whether a restrictive antibiotic regimen can decrease the prevalence of resistant isolates.

The plasmid type and antibiogram showed a high degree of correlation as has been reported by others [15,16,18,19]. Antibiogram determination combined with cluster analysis of zone sizes is much more simple than plasmid typing and can be performed in almost any microbiological laboratory as a rapid method for screening relatedness of strains. Use of different classes of antibiotics is advocated in order to prevent bias caused by cross-resistance between antibiotics used.

The present study shows a rapid colonization of neonates in the NICU with one type of a multiply resistant *S. epidermidis*. The phenotypic typing methods used do not allow differentiation between clonal or plasmid spread. This remains to be elucidated with genotypic typing methods.

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